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VITAL TOOLS

A BRIEF HISTORY OF CHO CELLS



Hamsters were instrumental in the development of molecular biology during the second half of the twentieth century, literally. Females imported from China donated ovary cells that enabled academic scientists to overcome technical obstacles and make early progress in the study of mammalian genetics. Later, the cells enabled industrial scientists to overcome technical obstacles and make early progress in the production of recombinant proteins. Today, Chinese hamster ovary (CHO) cells remain indispensable tools in both science and industry, and they may help translational scientists, pharmaceutical developers, and bioprocess engineers solve intractable problems in twenty-first century biomedicine and healthcare economics.

In the late fall of 1948, the Chinese civil war was approaching its climactic final scenes. As Mao Tse-Tung's communist forces marched across the country's northern provinces, a truck carrying a nondescript crate made its way from Peking to the republican capital of Nanking. The crate contained twenty compartments lined with wood shavings; each housed a Chinese hamster. There were ten males and ten females.

The hamsters were a gift from Dr. H.C. Hu of the Peking Union Medical College to Dr. Robert Briggs Watson, an American physician studying malaria in Asia for the Rockefeller Foundation's International Health Division. Watson was retrieving the animals for Victor Schwentker, a skilled rodent breeder in upstate New York. Schwentker had learned that the hamsters were valuable in biological and biomedical research. He also knew that it would be impossible to procure them after the Communists came to power.

On December 6, the hamsters were delivered to Watson's doorstep. Nanking was being evacuated. Only the Yangtze River separated the city from the Maoists. Watson was preparing to flee, while suffering from dysentery and a respiratory infection. On December 10, he packed his laboratory equipment into a station wagon. He packed the hamsters as well.

Against the advice of Chinese friends and the American Embassy, he braved an eleven-hour drive through blinding rain, first to Wuxi and then on to Shanghai, narrowly avoiding mudslides and roving bands of Communist troops, as the hamsters chattered away in their compartments.

The hamsters escaped China on December 12, 1948, on one of the last Pan-Am flights out of Shanghai. After the Maoists claimed victory and established the new People's Republic, Watson was accused of "war crimes" by the Chinese Germ Warfare Commission and tried in absentia for conspiring with Chinese nationalists on behalf of the US government to carry out a biological attack. H.C. Hu was also charged. He was convicted and sent to a detention camp for six months of "reeducation."

The hamsters landed in San Francisco, and were shipped to Schwentker's farm in New York. More than six decades later, cell lines originating from Hu's hamsters continue to serve as important tools in biomedical research and living factories for the manufacture of life-saving drugs.

Robert Briggs Watson in the 1920s



A RELUCTANT LAB ANIMAL

In 1919, Dr. E.T. Hsieh of the Peking Union Medical College became the first researcher to bring Chinese hamsters into the laboratory. He needed animals to inoculate, in order to distinguish strains of disease-causing pneumococcal bacteria. Mice were scarce, but hamsters were abundant in the fields surrounding Peking.

Five years later, Jocelyn Smyly, an Irish doctor working at the college, and American colleague Charles Young showed that Chinese hamsters were easily infected with the protozoan parasites that cause leishmaniasis (black fever). Soon, researchers throughout China were using captured Chinese hamsters to study a range of infectious diseases including tuberculosis, influenza, diphtheria, and rabies.

Unfortunately, the rodents couldn't be bred in captivity. Dr. Marshall Hertig made several attempts at Peking Union beginning in 1928, while he worked with Smyly and Young on leishmaniasis. When he left, he shipped 150 hamsters to the United States to establish a colony at the Harvard Medical School.

The attempt was an abysmal failure. The animals survived the bitter New England winter, but did not reproduce. Hertig built natural mating burrows in the basement of Harvard's Comparative Pathology building, and later in the grassy yard outside, but to no avail.

Scientists did not give up trying to domesticate and breed Chinese hamsters. They recognized that the hamster was an exceptionally useful animal model for genetic research. They become sexually active at two months, and their gestation period is only three weeks. Several generations could be studied in a single year.

In 1943, Italian geneticist Guido Pontecorvo came up with another good reason for using them. He spread metaphase hamster cell nuclei on microscope slides and—with the low-resolution instruments available to him at the University of Glasgow's Department of Zoology—counted fourteen large chromosomes.



Pan-Am routes in the Americas and across the Pacific in 1947

WATSON'S DIARY

December 1948,
Nanking to Shanghai

HW 148 178

Back at the laboratory I ran into CKO, who was coming over to see me. When we got to the laboratory I found everyone busily engaged in packing up various supplies, some things I planned to get to Taiwan, if possible. I stopped this and learned that they were only carrying out literally Han's suggestion that they take anything they

Monday, 6 December 1948

A busy day and one made not less complicated and glad by the unexpected receipt of 20 hamsters from Peiping for forwarding to New York. I had forgotten the request that had gone up to Dr. Hu at PUMC some weeks ago, for the animals. They came down in an AAG courier plane, travelling almost in solitary splendor: the other passengers were 2 dachshund puppies. Am keeping the hamsters in my bedroom, away from the dog, and they seem in good spirits.

for him to get a ticket.

To the laboratory, to meet Miss Han and Chang, who has decided to go to Taiwan. Made a check list for them to follow. Will try to get things airtight to Taipei or Ping Tung via AAG.

Nanking

Monday, 6 December 1948

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Packed and delivered for air shipment, AAG, 7 cases of optical and other equipment all of JCC's engineering instruments, note book, for the flights go only on routine and are about to stop. Packed cases into my storeroom. Our room is the first place and I know what is going on our supervision of it ends.

HW 148

until they could be loaded. There was no way of determining how long I would have to wait. I talked with the packers and they said that they could pack my things, but this had to be done in the yard in the rain and they had no tarpaulins. They wanted to see what they could do.

Fred Schultheiss of the Embassy called in the evening to say that he would advise against driving to Shanghai because roving bands of communists were across the Yangtze between Nanking and Wuhsi and might stop me. They had fought two minor engagements along the railway today. These men are thought to be irregular elements of Chen Yi's forces that have crossed to upset the countryside. I told him that I would chance the trip and he then said that tomorrow would probably be the last day I would have a chance to drive through. He warned against driving at night.

Friday, 10 December

The station wagon being loaded last night, we set out from 16 Ch'ih Pi Lu at 5:45. The wagon was heavily laden and I was fearful that it was overloaded, but couldn't decide what to take off and set out anyhow, armed with a bottle of brandy, a book of common prayer and my old .45 Colt. It was black dark and very few people were on the streets although curfew ends at 5:00.

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Saturday, 11 December

The hamsters made the trip just fine and wakened me with their chattering. They are off to the States tomorrow, so CGC and I changed their sawdust and examined each one to make sure each was well. Besides, I had to sign a certificate of health required by the Chinese customs for some reason not clear to me. Judging by the way they ate black beans and kaoliang (millet) and chatter all night, they are in fine health. They are cunning little animals

HW 148 179

CKO came in for a talk and I told him that someone would have to arrange the details of assignment of the two groups of salaried workers we have trained. Han simply told them they were going to Chongshan and Chungking, without making any arrangements. We will give them what equipment and supplies we have, but we can't furnish optical equipment.

Saw Dr. Tsaur, at his request. He is very much upset by the move of JCC to Canton without warning. He feels that CKO cannot be of much use to the program and has asked CKO and IGY to resign from the advisory committee. He had known nothing of Han's leaving and says that the matter of his transfer has not been formalized; in general he seemed nettled by the lack of formal relationships between JCC and NIH. Gave him a case of beer and this may make him feel better.

Tuesday, 7 December

Slept poorly from coughing and from the hamsters chattering and gnawing at their cages. Back at the NIH most of the day, getting things packed up. Have given the two units some expendable supplies that we cannot move. They are packing up with great confidence that they will get off to the west, but no-one will venture as to how this will be done.

Had a long talk with CKO. He thinks that most government offices will move somewhere. He has sent most of the parasitology laboratory to Chakiang and from the looks of things, most of the NIH's is packing to go somewhere. Says that he has heard from Yen, who welcomes the NIH to Taiwan; what do I think? I told him I thought the idea a poor one, though one unit might go in connection with supervision of biological production.

HW 148 181

We reached the east gate as one of the three great gates swung open at 8:00 and were not stopped for a check. I drove for the first hour and we made 40 kilometers in this time. Day broke over the black countryside during this hour. The country people gather grass for fuel and at this season there is very little left to be seen. There was little traffic on the road, but now and again we passed convoys of wheelbarrows, each complaining to high heaven; I never knew a Chinese wheelbarrow that didn't squeak.

The trip was uneventful until we reached Wuhsi, when Wang insisted on taking the road to Nanpohow. The Chinese road signs give only the name of the next place and Wang insisted that we didn't actually go through Wuhsi, which I know we did. This put us 40 minutes behind schedule and nearly broke a spring, since when we turned around Wang tried to make time over a road that was not made for speed. Wang is a city driver, rarely out of Shanghai. He has great contempt for anything or anyone not of Shanghai. He asked directions in Wuhsi and he asked me if I had understood the directions, which I had done with no difficulty. He laughed and said "These Wuhsi people speak such crazy Chinese I thought you couldn't understand." Shanghai dialect is the worst language I ever heard and Wang adds to its general difficulty by being tongue tied.

We stopped for checking at Wuhsi, Suhsow and Kiating, the latter just out of Shanghai. The official chop of the garrison commander at Nanking served us in good stead and kept down a lot of talk.

Tai Hu, the great lake near Suhsow, was lovely and covered

Mice have forty. Rats have forty-two.

The size and low number of the hamster chromosomes facilitated cytogenetic research. Given the methods of the day, they were the easiest rodent chromosomes to identify, characterize, and map. Geneticists came to covet the animals, and persisted in breeding experiments.

HAMSTER WHISPERERS

Victor Schwentker decided to try his hand, too. He had a thriving animal supply business in Brant Lake, New York, seventy miles north of Albany. He bred mice, rats, voles, moles, rabbits, hamsters and guinea pigs. By 1948, he had become the largest supplier of animals to biological laboratories in the northeastern United States.

Schwentker knew that demand for Chinese hamsters would be high. He found Robert Briggs Watson in China, through contacts among his biomedical research customers, and arranged to have some of the animals shipped to the United States.

Where others had failed, Schwentker managed to domesticate and breed the creatures in captivity. The process entailed a great deal of labor intensive taming. Within two years, Schwentker had a thriving colony, the first established outside of China. Word spread, and researchers started placing orders.

George Yerganian, a graduate student at Harvard, was one of them. He was conducting doctoral research on plant genetics, but in 1948, he found Pontecorvo's paper in a Harvard Library, and realized that the hamsters' low chromosome count would make the species a preferred experimental model. He purchased several animals in order to study their estrous cycles and mating habits.

In 1951, Yerganian began working on a postdoctoral fellowship in radiation biology at the Brookhaven National Laboratory on Long Island. He gained access to microscopes more powerful than those used by Pontecorvo and determined the correct number of chromosomes in Chinese hamsters: twenty-two. Two other cytogeneticists reached the same conclusion independently, Robert Matthey at the Université de Lausanne in Switzerland, and Leo Sachs at the John Innes Institute in Norwich, England.

Schwentker discontinued sales of Chinese hamsters in 1954. They were popular with researchers, but the animals are naturally solitary, and females became aggressive in captivity. Raising and breeding them was difficult and laborious. Schwentker



A Chinese hamster

DIABETIC HAMSTERS

In the late 1950s, George Yerganian noticed that repeated inbreeding produced lines in which the hamsters uniformly developed symptoms of adult-onset diabetes, including periodontal, pancreatic, and retinal problems. For the next decade, he published papers on these and other pathologies with collaborators from Boston area hospitals and universities, and widely distributed diabetic hamsters to research laboratories and pharmaceutical companies in Europe and North America. Teams at the Upjohn Company of Kalamazoo, Michigan and the Charles H. Best Institute in Toronto bred the animals and established new colonies. Eventually, biomedical researchers around the world adopted the Chinese hamster as a standard animal model in which to study the appearance and progression of spontaneous diabetes. Yerganian's Boston University vivarium was the point of origin.

never published or shared his breeding techniques, but by 1954, Yerganian had devised his own hamster-taming methods.

Yerganian had accepted a joint appointment the year before at Boston University and the Children's Cancer Research Foundation (which later became the Dana-Farber Cancer Institute). With funds granted by the National Cancer Institute, he established a breeding center and began distributing hamsters to scientific colleagues. For the next decade, he was the sole supplier of Chinese hamsters to biomedical research institutions in the United States.

In 1983, Yerganian launched a private company, Cytogen Research and Development, Inc., to supply Chinese hamsters to public, non-profit, and commercial research laboratories. For many years, the company's facilities were located on the Brandeis University campus in Waltham, Massachusetts.

"THE MAMMALIAN *E. COLI*"

In the 1950s, studies in human and animal genetics were hindered by a lack of mammalian cell lines. Researchers had tried for decades to grow *ex vivo* animal cell cultures, but cells typically survived for just a few division cycles. Efforts to generate and maintain continuously growing mammalian cell lines ended routinely in failure and frustration. Contamination by bacteria and molds was common, but even when this problem was solved in the 1940s by the introduction of antibiotics, the long-term viability of animal cell cultures did not improve.

There were exceptions. In 1943, Wilton Earle and colleagues propagated the first continuously growing mammalian cell line, mouse L, at the National Cancer Institute, and in 1951, Dr. George Gey grew the first immortal human cells, the famous HeLa line, at the Johns Hopkins University School of Medicine. But these cultures were mixtures of heterogeneous cells, many of which contained chromosomal abnormalities. For many inquiries, Mouse L and HeLa cells had limited utility.

Important advances were made in 1948 when

Dr. Theodore Puck



Earle's lab established a clonal (genetically homogenous) mouse L culture, called mouse L929, and in 1955, when geneticist Theodore Puck managed to isolate and propagate single clones and establish clonal HeLa cultures at the University of Colorado School of Medicine in Denver.

Researchers in Puck's laboratory went on to develop novel *in vitro* culturing techniques, special growth media for mammalian cells, a large collection of useful human and animal cell lines, and methods for mutagenesis and gene mapping that enabled—for the first time—studies of molecular genetics in mammalian cells.

In 1957, Puck learned of the Chinese hamster and its compact genome. He contacted George Yerganian and asked for specimens. Yerganian sent a single adult female, housed in a handmade box with a mesh top. She arrived by railway courier, after riding trains for several days. No one could have predicted how important this single hamster would become in the history of the life sciences, biomedicine, and the biopharmaceutical industry.

Puck removed an ovary, extracted a cell, and gently coaxed it into expansion in a petri dish. It was the first culture derived from a Chinese hamster. Puck found that with proper treatment, CHO cell cultures grew quickly. The cells were hardy and could be maintained indefinitely. By subcloning, Puck and a junior colleague, Fa-Ten Kao, generated the CHO-K1 cell line, which became a standard research tool in molecular and cell biology.

Labs from around the world requested cells from Denver, and Puck distributed them freely. His CHO cell lines became the gold standard for *in vitro* studies of mammalian biology. He took to calling them “the mammalian *E. coli*.” Molecular genetics had previously advanced mainly through studies of microbes—viruses, bacteria, and fungi. After 1957, thanks largely to Puck and the unique properties of CHO cells, geneticists had new opportunities to study higher organisms.

ADD SUGAR

In 1973 and 1974, University of California, San Francisco biochemist Herbert Boyer and Stanford University geneticist Stanley Cohen conducted a series of experiments that demonstrated the utility and power of recombinant DNA technology as

an instrument of genetic engineering. The invention set the stage for the birth of the biotechnology industry.

Genentech, the first company established to commercialize the technology, made headlines in 1978 and 1979 when it reported the manufacture of medically useful peptide hormones, first insulin and then human growth hormone, in bacteria, in *E. coli*—naturally, because molecular biologists knew far more about *E. coli* cells than other any kind. They had been studying *E. coli* for decades. Researchers had developed an intuitive feel for its behaviors, proclivities, moods, and reactions.

Genentech's accomplishments were impressive, and they stirred competition. Many molecular biologists were encouraged to reproduce the company's success with other genes and molecules of commercial value. A host of biotech startups sprang up, endowed with sufficient capital to explore the potentially lucrative new field.

After insulin and growth hormone, Genentech selected the gene for tissue plasminogen activator (tPA) as a cloning priority. So did several competitors. tPA is a blood clot dissolving protein. It was considered a promising treatment for heart attacks. The size of the potential market made it an enticing target.

Genentech scientists isolated the gene and plugged it into the *E. coli* expression system that had produced insulin and human growth hormone. This time, the result was different. Dennis Kleid, one of the company's early cloners, reports that the bacteria made only half-hearted efforts to express the molecule. “Just tiny amounts were detected,” he says, “and the protein wasn't folded properly.”

The failure was a reality check. Researchers at Genentech and competing firms had hoped that simple prokaryotic cells would be suitable for the commercial production of a wide range of large, complex human proteins. The tPA experience gave them pause.

The post-mortem drew attention to the many post-translational modifications that cells make to turn amino acid chains into functional proteins. In human beings, the process is complex; prokaryotic bacterial cells don't possess the same modification repertoires. In the tPA experiment, the molecule hadn't folded properly because *E. coli* isn't fully equipped for mammalian glycosylation.

Glycosylation is an enzymatic process in which sugar groups are linked covalently to newly synthesized proteins. The sugars cause the proteins to fold into stable, soluble forms. Early experiments with *E. coli* and other microbes taught gene cloners that prokaryotic cells would not turn some heterologous (foreign) gene products into biologically active molecules. tPA was one of them.

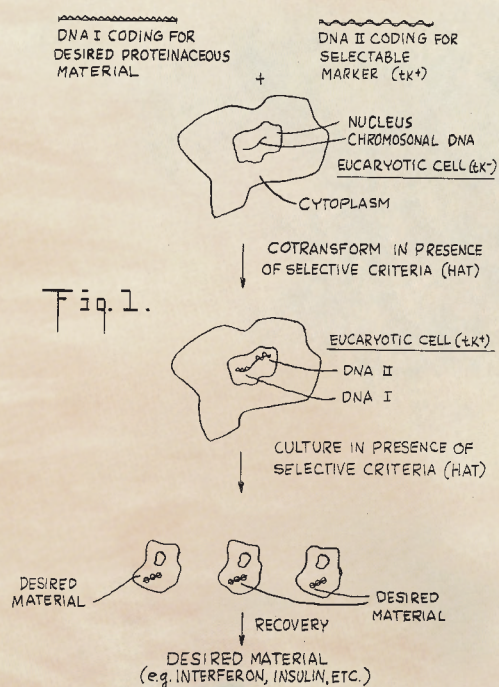
The use of *E. coli* as a recombinant protein factory gave rise to other sorts of problems. In 1980, for example, Genentech came to a dead end in its quest to make a recombinant hepatitis B vaccine. When company scientists inserted the gene for a viral antigen into *E. coli*, the bacterium's cellular machinery lurched to a halt. Dennis Kleid remembers the mishap: “*E. coli* hated that protein. The bacteria stopped growing. They just quit.”

THE “AXEL PATENTS”

On February 25, 1980, Columbia University inventors, molecular biologist Richard Axel, microbiologist Saul J. Silverstein, and geneticist Michael H. Wigler, filed an application with the United States Patent and Trademark Office (USPTO) for a patent on the “Wigler method” of “co-transformation,” techniques for cloning and expressing heterologous genes in nucleated eukaryotic cells. The Cohen and Boyer invention had described the re-engineering of non-nucleated prokaryotes, such as bacteria. The Wigler method became a standard tool in mammalian biology and genetics, biomedical research, and commercial biotech manufacturing. The USPTO issued the initial patent to Columbia University in 1983. The claims were broad. They covered many different vectors and cell types in the production of many different recombinant proteins, and the university made nine additional filings to extend, refine, and manage the patent estate. Ten firms, including Amgen, Biogen, Genentech, and Genetics Institute, purchased licenses at low “early bird” prices. After June 1, 1984, the university granted twenty-four additional licenses at a higher rate. By the time the patents expired in August 2000, they had generated more than \$790 million in royalties.

U.S. Patent Aug. 16, 1983 Sheet 1 of 2 4,399,216

COTRANSFORMATION OF EUKARYOTIC CELLS



The problem stemmed from the fact that *E. coli* does not secrete proteins in large quantities. *E. coli* is a gram-negative bacterium. Its envelope has two membranes, each with different properties and functions. Genentech found that recombinant proteins generally don't cross both of these barriers without assistance.

Consequently, recovery of proteins from *E. coli* entailed lysing the cells, which complicated the purification process and added to production costs. And in the case of the hepatitis B project, the presence of hepatitis antigen in the cytoplasm evidently caused enough discomfort that the cells stopped dividing.

The problems with *E. coli* prompted Genentech to hire Arthur Levinson, a postdoc at the University of California, San Francisco, to investigate eukaryotic expression systems. Levinson worked first to express the hepatitis antigen in yeast, and then he turned to mammalian cells. By August 1981, he had developed an experimental expression system in monkey kidney fibroblasts.

AMPING UP

Mammalian cell expression was a wide-open field, but Levinson wasn't the first entrant. By the time he began experimenting with monkey kidney cells, Michael Wigler, Richard Axel, Saul Silverstein, and colleagues at Columbia University had already been putting recombinant DNA into mouse cells for nearly three years. In 1979, they showed how to clone and express genes coding for desired proteins along with selectable markers. They filed a patent on the invention in February 1980.

But protein yields in early mammalian cell expression systems were disappointing. Research conducted several years before in the laboratory of Stanford biologist Robert Schimke provided means of improvement. In 1976, as Schimke was studying how cancer cells develop resistance to chemotherapeutic agents, one of his graduate students, Fred Alt, discovered a phenomenon called gene amplification.

Alt observed that when mouse sarcoma cells were exposed to methotrexate, a highly toxic cancer drug, most died but some became resistant and survived. He and Schimke investigated and found that methotrexate inhibits a vital enzyme called dihydrofolate reductase (DHFR). Somehow, resistant cells made tens or even hundreds of copies of the DHFR gene, which produced enough excess DHFR to overcome the methotrexate in the medium.

In their patent application and related papers, Axel and colleagues at Columbia proposed that DHFR amplification could significantly increase gene expression in mammalian cells. A test of the idea became feasible in 1980, when Columbia cell biologists Lawrence Chasin and Gail Urlaub isolated mutant CHO cells that lacked the enzyme.

In 1982, a postdoc working in Phil Sharp's MIT laboratory had the idea of engineering these DHFR-deficient cells for the production of recombinant proteins. Randy Kaufman had been a graduate student in Schimke's lab at Stanford. He spliced the

BLOCKBUSTER DRUGS MADE IN CHO CELLS

Product	Sales (USD B)	Year of first Approval	Patent Expiry (US)	Company
Humira (anti-TNF)	11.00	2002	2016	AbbVie; Eisai
Enbrel (anti-TNF)	8.76	1998	2028	Amgen; Pfizer; Takeda
Rituxan/MabThera (anti CD20)	7.91	1997	2016	Biogen Idec; Roche
Avastin (anti-VEGF)	6.97	2004	2017	Roche/Genentech
Herceptin (anti-HER2)	6.91	1998	2019	Roche/Genentech
Epogen (epoetin alfa)	3.35	1989	2013	Amgen; Johnson & Johnson
Avonex (IFN- β -1a)	3.00	1996	2015	Biogen Idec
Rebif (IFN- β -1a)	2.59	1998	2013	Merck Serono
Aranesp/Nesp (darbepoetin α)	2.42	2001	2024	Amgen
Advate/Recombinant (Octocog α)	2.37	1992		Baxter
Eylea (anti-VEGF)	1.88	2011	2021	Regeneron; Bayer Healthcare
Total Sales in 2013	57.16			

DHFR gene into an engineered plasmid (a circular ring of DNA) adjacent to a gene that codes for a monkey virus (SV40) protein, and then introduced the plasmid into the DHFR-deficient CHO cell mutants.

He anticipated being able to select for cells that both survived exposure to methotrexate and produced the SV40 protein in large quantities—if, as he hoped, the cells generated copies of both linked genes. It worked. In fact, the genes from the plasmid were incorporated and amplified as part of the hamster genome. It was possible to increase yields.

On March 23, 1982, Kaufman and Sharp submitted an article on the work to the *Journal of Molecular Biology*. Kaufman subsequently constructed a vector for amplified expression of alpha interferon in CHO cells. He recalls encouraging Sharp to file for a patent on the invention, but the lab chief declined.

As a co-founder of Biogen—which was at the time working to develop alpha interferon as a pharmaceutical product—Sharp recognized the value of amplified gene expression in cells that could fold human proteins into proper shape, but he was satisfied that the Axel patent had wrapped up the territory. As it happened, Biogen didn't use Kaufman's system. The company's manufacturing and marketing partner, Schering-Plough, made interferon in *E. coli*.

By the beginning of 1983, Genentech's Levinson had also devised a DHFR expression system with help from Chris Simonson, another alumnus of Schimke's lab. The pair filed a patent application on January 19. Later in the year, Kaufman took his CHO cell expertise to Genetics Institute in Boston, where he worked on the production of tPA, erythropoietin (EPO), a red blood cell growth-stimulating hormone, and Factor VIII, a blood clotting factor.

SCALING UP

Success in boosting CHO cell expression created a new set of problems. Once companies learned how to make proteins in CHO cells, they had to install manufacturing processes. Mammalian cell cultures had never been grown on industrial scales. In the early 1980s, it was generally assumed that they were not well suited to growth in suspension in fermentation tanks. CHO cell manufacturing became a technological adventure.

Growth media in high volume bioreactors are stirred in order to maintain optimal or at least workable environmental conditions—temperatures, pH levels, oxygen transfer rates, broth consistencies, and cell densities, for example. Precision control is necessary to achieve efficiency and quality in production, but stirring creates turbulence in growth media and shear forces greater than fragile mammalian cells can withstand.

In the early 1980s, all good cell biologists knew that mammalian cell cultures grew best in roller bottles. Roller bottles are small vessels that contain liquid cell growth media. Cell cultures coat the interior surfaces in a thin monolayer, and the bottles are slowly rotated. The action alternately washes the cells in the growth medium and exposes them to air.

Genentech and other early biotech companies used roller bottles to grow mammalian cells that expressed and secreted functional recombinant human proteins, but when it came time to scale up, they had no blueprints to follow. No one had ever assembled an industrial scale mammalian cell culture production system. Company scientists had no idea how adaptable or scalable their processes would be, if at all. Everything was experimental.

Genentech ventured first into this uncharted territory as it prepared to introduce CHO cell-derived tPA. The initial task was to produce enough of the drug to supply clinical trials. Bill

Young was Genentech's head of manufacturing. He remembers that it was difficult to project demand—no one knew how much of the drug to administer to patients.

The trials began with very low doses, but the clinicians kept bucking them up. Young worked with calculations that started at 5 milligrams per dose and rose to 150 milligrams. Soon, he says, "It was almost impossible to make enough product in roller bottles. I could envision these bottles taking off over the entire building. It was the Rube Goldberg approach to biotech manufacturing. We just kept adding more and more bottles." Genentech had a problem.

Young credits Jim Swartz with casually ushering in a new era of bioprocess engineering. Swartz was a chemical engineer who had come into the company from Eli Lilly and Company. According to Young, he asked, as the manufacturing group mulled over its predicament, "Why don't we try growing these cells in a fermenter? We've got a 10,000-liter fermenter that we bought for bacterial work. How do we know that the cells won't grow in it?"

The cell biologists on staff repeated the conventional wisdom that mammalian cells are too delicate, but Dennis Kleid remembers a timely and influential suggestion from a contrarian, Rob Arathoon: "He suggested that we change the gear ratio on the impeller and stir the tank very, very slowly." Some calculations indicated that gentle agitation could work for CHO cells, which grow relatively slowly and need less oxygen than *E. coli*. The idea gained traction.

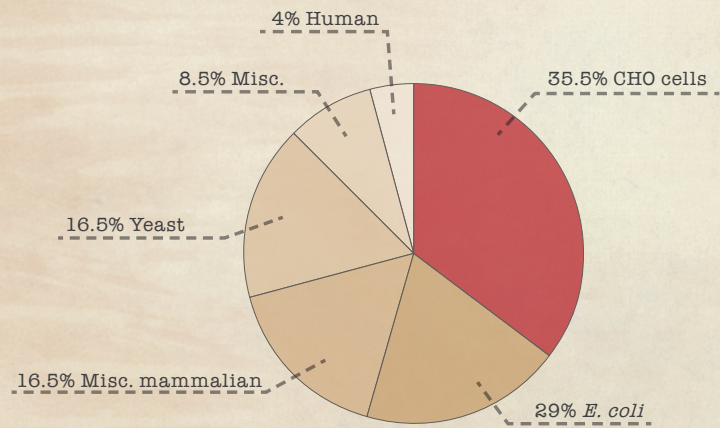
Levinson worked with the company's manufacturing group to design a bioprocessing system that would facilitate the growth of genetically engineered CHO cells in suspension in the 10,000-liter bioreactor. Young hired three industrial microbiologists from Burroughs Wellcome with experience in large-scale cultures for animal vaccines, and the project team worked through a host of technical and regulatory issues—purification, validation, risks of viral contamination, and so on—to deliver clinical grade tPA.

Young calls the scale up process "horrendous," but somehow it all came together. In fact, the company discovered that CHO cells in suspension made a more potent product. When the big bioreactor went online, the medical staff had to back down recommended doses from 150 to 100 milligrams. It was a mystery. "The molecule was different chemically," says Young, "but we never found out exactly why."

On November 13, 1987, Genentech's tPA became the first FDA-approved pharmaceutical product manufactured in CHO cells. The commercial performance of the product was underwhelming, but the design and construction of the manufacturing system was genuinely innovative. "Nobody had ever done anything like this," says Young. "It was a completely new way of making a pharmaceutical product."

In the mid-to-late 1980s, many companies followed Genentech's lead and turned to eukaryotic cells as *E. coli* systems proved inadequate or inferior to viable alternatives. CHO cells

RECOMBINANT DNA EXPRESSION SYSTEMS



Biopharmaceutical applications (1982 to 2014)

became, and they remain, preferred hosts for the production of recombinant protein therapeutics. Of the twenty top-selling biopharmaceuticals on the market in 2013, eleven were manufactured in CHO cells. Combined annual sales of these protein products exceeded \$57 billion.

BIOPROCESS OPTIMIZATION

On May 2, 2014, Biogen CEO George Scangos delivered the annual Michaels Lecture to the MIT Department of Chemical Engineering. He spoke about challenges and opportunities in bioprocessing and observed that since the 1980s, yields and costs in protein drug manufacturing have followed a biological analogue of Moore's Law.

In 1965, Intel co-founder Gordon Moore predicted that transistor counts in integrated circuits and computing capacities and speeds would double every two years, and so they have. This year, Scangos explained to his MIT audience that outputs in biotech manufacturing have also increased exponentially. Cell biologists and bioprocess engineers have boosted cell culture yields from 100 milligrams per liter in the early 1980s (at a cost of \$10,000 per gram) to 5 grams per liter today (at \$100 per gram).

Advances in computing are now approaching physical barriers. Soon, it will be impossible to go smaller. According to Scangos, advances in bioprocess manufacturing are also pushing up against limits—not physical, but economic. Biogen currently controls 10 percent of the world's mammalian cell culture capacity (Roche, Amgen, and Biogen together account for 55 percent of the total). Scangos believes that it has become infeasible for biotech manufacturing operations to continue leveraging classical economies of scale.

There are some further parallels. Physicists and electrical engineers contend that after the end of Moore's Law, advances in computing power will result from greater energy efficiency and the emulation of materials and architectures in biological information processing systems—DNA, cells, and brains, for

example. Similarly, Scangos maintains that future advances in bioprocessing will be realized through improved utilization of existing assets and the installation of flexible, networked communication, supply, and production processes.

On the technical side, cell biologists and bioprocess engineers continue to improve the utilization of the industry's vital tools. They are learning how to make better bioreactors and growth media, and how to make cells healthier, happier, and more sociable—i.e., tolerant of increasing cell densities—in order to improve protein yields. Drug companies have developed a wide variety of CHO cell lines with genotypes, phenotypes, and behaviors adapted for expression of different kinds of recombinant proteins.

HAMSTER CELL GENOMES

In the age of genomics and bioinformatics, these conventional efforts are swimming in great pools of new data. Researchers have gained access to the astoundingly complex universe of molecules and pathways that constitute mammalian cell drug factories. Nate Lewis, a systems biologist and assistant professor at UC San Diego School of Medicine who studies CHO cells, says, “In the past, researchers tweaked environmental conditions, or the vector with the inserted gene, but they never really knew what was going on inside the CHO cell itself. The cell was a black box.”

To open the black box, several big biotech and pharmaceutical companies launched a private consortium to sequence the genome of several CHO cell lines in 2006. In a former life as an industrial scientist, Lewis became part of a second sequencing effort initiated by GT Life Sciences, because, he says, “The lack of a genome had really stalled CHO research. After the *E. coli* genome was sequenced, researchers were able to do metabolic engineering in bacteria. We wanted to be able to do it in CHO cells.”

Scientists are now using CHO genome sequences to provide detailed portraits of transcription, translation, protein synthesis, and post-translational modification in altered hamster cells. They are uncovering biomarkers that distinguish high-yield lines, and others that point to rate-limiting cellular processes.

They have shed light on hamster glycosylation pathways.

Vanishingly small variances in glycosylation can have dramatic impacts on drug efficacy, stability, and safety. The biochemical toolkit utilized by CHO cells resembles that found in human cells, but hamsters lack a few key enzymes, and they possess others that add non-human sugar modifications, which may diminish therapeutic efficacy or induce immune responses. All of this is grist for the molecular pharmacologist's mill.

Drug makers are now combining their expanded knowledge base with precision tools for knocking genes down and out. In 2009, Alnylam of Cambridge, Massachusetts began applying RNA interference (RNAi) technology to silence select CHO cell genes. In May 2014, researchers at the Technical University of Denmark made the first demonstration of CHO genome editing with CRISPR/Cas9 technology. The targets included genes that diminish the efficacy of therapeutic antibodies.

Others researchers are attempting to create humanized CHO cell lines by knocking out CHO genes that code for enzymes involved in non-human modifications. Such an approach could enable CHO cells to mimic human glycosylation, and drug makers to produce safer, more efficacious medicines.

At UCSD, Lewis is taking a global “systems biology” view of CHO cell protein factories. He is using computational methods to build models of metabolic pathways involved in recombinant protein secretion. He has two goals. He wants first to generate predictive rules that researchers can use to determine optimal cell lines, media, and growth conditions for the high volume manufacture of specific proteins.

Secondly, he wants to develop predictive algorithms for engineering cell lines, biological systems that will produce high quality biotherapeutics displaying a wide range of desired characteristics, properties, and specificities. “With the genome sequence and these models in hand,” he says, “we will be able to control in a very specific manner the attributes of proteins.”

CHO CELLS, DRUG PRICES, AND BIOSIMILARS

The metabolic engineering of mammalian cells can enhance the safety and efficacy of new biological drugs. It may also further improve biomanufacturing processes, reduce production

SEQUENCING THE CHO GENOME

When plans for the CHO cell genome project were announced, many researchers hoped that it would lead to deeper understandings of CHO biology. They envisioned the creation of “designer” cell lines and vaulting advances in biopharmaceutical development. This promise has not yet been fully realized, but the field is moving rapidly from the manipulation of single genes to multiple gene orchestration.

In 2006, leading biotech and pharmaceutical companies joined forces with the Society for Biological Engineers to establish the CHO Consortium. Member organizations worked cooperatively to map and sequence the genomes of several different CHO cell lines, and agreed to share resulting

intellectual properties. Members had full access to the consortium's extensive database. Participating companies included Bayer Healthcare, Boehringer Ingelheim, Bristol-Myers Squibb, SAFC Biosciences, and Schering Plough.

GT Life Sciences, a privately held San Diego firm, launched a second effort to sequence CHO cell line genomes, in partnership with the Beijing Genomics Institute (now BGI). In August 2011, the partners published the first open access CHO genome sequence, for the CHO-K1 cell line. Two months later, GT Life Sciences was acquired by Intrexon Corporation, a synthetic biology company located in the San Francisco Bay Area.

costs, and perhaps help to lower drug prices—at a time when healthcare expenditures are spiraling upwards and payers are exerting intensified pressure on drug companies to engage in deep discounting.

The biotech sector is at the center of the imbroglio because it has focused largely on the development of innovative specialty drugs and treatments for unmet medical needs. Branded biopharmaceuticals are often the best and sometimes the only treatment options available to doctors and patients, and in the age of genomics and precision medicine, these high value products frequently serve small patient populations. The prices are very high.

The economic pain has led payers—patients, providers, insurance carriers, pharmacy benefits managers, governments, taxpayers, employers, and labor unions—to question the value of the products. Are the benefits worth the great expense? When the question was put to Severin Schwan, CEO of the Swiss pharmaceutical company, F. Hoffman-LaRoche, he said, “There is no objective answer. At the end, you are discussing, what is the price of life?”

Schwan implies that the issue is for society to decide, not CEOs, accountants, or scientists. How much should we pay to take care of people? How much can we afford to pay? Who should decide, and on what basis? Schwan also implies that the prices are fair: the drugs give “life” and society at large must decide whether to pay for it.

Finally, he assumes relations of trust between corporations and communities, but these seem to have broken down. In public debates on pricing, drug companies are portrayed, in turn, as ethical firms doing their best to balance obligations to customers and shareholders, and as price gougers motivated by unbridled greed.

Industrialists maintain that the high prices reflect the realities of drug development: it is immensely difficult, enormously expensive, intensely competitive, and highly regulated. The vast majority of projects miscarry. Revenues from successful products must subsidize a host of failures. Innovation is a risky, costly business.

In November 2014, The Tufts University Center for the Study of Drug Development (CSDD) released an estimate of total development costs for a new FDA-approved pharmaceutical product in the United States: \$2.6 billion. Critics are loath to accept the figure. Rohit Malpani, policy director of Doctors Without Borders told *The Economist*, “If you believe that, you probably also believe the earth is flat.” Skeptics complain that Tufts relied on information supplied by pharmaceutical companies.

The debate is heating up, but process improvements are unlikely to have substantial impacts on prices for patented biopharmaceuticals, because manufacturing costs represent only a small fraction of total expenditures. But even slight savings could make a difference in markets for generic products, where producers compete on the basis of price.

In the United States, biopharmaceutical manufacturers have not yet faced competition from off-brand products, but many first generation protein drugs will soon lose patent protection. A host of companies are gearing up to develop facsimiles.

The products are called “biosimilars” or “biological

CELL-FREE GENE EXPRESSION AND PROTEIN SYNTHESIS

Genetically engineered CHO cells may represent the future of biopharmaceutical production. Or they may have no future at all. Sutro Biopharma would prefer the latter outcome. The company is developing a cell-free gene expression platform as an alternative to *in vivo* cell culture and transgenic modes of production. The idea is to transcend limitations imposed by adapted biological systems. Sutro’s alternative separates all cellular components required for transcription, translation, and protein synthesis into an extract. Users simply add DNA encoding desired proteins. The company claims that its technology optimizes biochemical processing and affords grams per liter yields in just eight to ten hours even at commercial production scales.

follow-ons” rather than generics because they resemble the original products, but are not identical. The complexity of biological molecules precludes the design of exact replicas. Protein products can vary from factory to factory even if the same host cells and manufacturing process are used.

Market penetration of biosimilars could be rapid in the United States if the products offer significant savings. A report published last year by the RAND Corporation estimated price cuts between 10 and 35 percent. Competition among small molecule generics typically cuts prices in half, but the biologicals will have higher production costs. They may also be required to clear significant regulatory hurdles.

The regulatory environment is unsettled. The 2010 Patient Protection and Affordable Care Act created an abbreviated licensing pathway for biological products that are “interchangeable” with licensed drugs, but the FDA is still finalizing regulations. If the rules favor developers, healthcare payers will surely gravitate to the cheaper alternatives.

Elsewhere around the world, the picture is clearer. The European Union established an approval process in 2004, and the first wave of products appeared two years later. As of May 2014, twenty products had been approved for sale. Investment in biosimilars production is also growing rapidly in China and India, where high-cost branded biologics strain national healthcare systems. Demand is high for alternatives, and regulatory barriers are relatively low.

CHO cells have served as vital tools in the development of innovative protein therapeutics for more than thirty years. Now, they are helping drug makers deliver follow-ons at affordable prices to doctors and patients in more than fifty countries. If molecular biologists can engineer CHO cells for more efficient cell culture production, the result will be better medicines at lower costs for millions of people around the world. ■

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